CONCISE REPORT

 β_2 Adrenoceptor gene single nucleotide polymorphisms are associated with rheumatoid arthritis in northern Sweden

B-Y Xu, L Ärleth, S-B Rantapää-Dahlquist, A K Lefvert

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Background: β_2 Adrenoceptor (β_2 -AR) represents a link between the sympathetic nervous system and the immune system, and may be involved in human rheumatoid arthritis (RA). The gene encoding β_2 -AR contains three single nucleotide polymorphisms (SNPs) at amino acid positions 16, 27, and 164.

Objective: To examine the common variants at positions 16 and 27 and their association with RA.

Methods: An allele-specific polymerase chain reaction to determine the common variants at positions 16 and 27 was used in 154 patients with RA and 198 ethnically matched healthy subjects from northern Sweden.

Results: Carriage of Arg16 and of Gln27 was associated with RA. Carriage of Gln27 was associated with activity of the disease and in combination with non-carriage of Arg16 with higher levels of rheumatoid factor.

Conclusion: The β_2 -AR SNPs may thus constitute an additional non-major histocompatibility complex association in RA.

promoted by agonists was enhanced in receptors homozygous for glycine16 (Gly16) as compared with those homozygous for arginine16 (Arg16). Receptors homozygous for glutamic acid 27 (Glu27) were resistant to such down regulation when compared with those homozygous for glutamine 27 (Gln27).¹⁹ The receptors homozygous for isoleucine 164 (Ile164) had markedly decreased ligand binding and coupling properties compared with those homozygous for threonine 164 (Thr164). Cardiac dysfunction was seen in transgenic mice which had the Ile164 form of β_2 -AR.²¹

Certain genotypes are associated with the concentration of IgE in the blood of asthmatic patients²² and with antibodies against the β_2 -AR in patients with myasthenia gravis.²³ Certain others confer susceptibility to asthma,^{24–27} hypertension,²⁸ obesity,²⁹ and myasthenia gravis.²³ The involvement of β_2 -AR in RA and the importance of β_2 -AR for regulation of the immune system imply a potential importance of genetic variations of this gene also in RA. This study examined the association between RA and SNPs at amino acid positions 16 and 27. The SNP at amino acid position 164 was not analysed, because the frequency of homozygosity for Thr at that position is >95% in the Swedish population.^{22 23 29}

MATERIALS AND METHODS

Study groups

One hundred and fifty four white Swedish patients with RA (American College of Rheumatology criteria³⁰), 42 men and 112 women, from the northern parts of Sweden (Västerbotten), and 198 ethnically matched healthy subjects, 138 women and 60 men, from the same area were studied. Levels of RF according to Waehler-Rose were tested in 144 patients; six patients lacked RF. The titres of RF were 1/40 to 1/40 000. The accumulated inflammatory activity of the disease was estimated by one score comprising the first 5 years of disease. The score was based on three grades for each of the erythrocyte sedimentation rate, number of swollen and tender joints, and the doctor's global assessment of disease activity, and was calculated at disease onset, and after 1, 3, and 5 years, respectively. The sum was divided by the number of observations, giving a score between 3 and 9.³¹ This activity score was evaluated in 153 patients, all of whom had an activity score between 3.0 and 8.0. One hundred and forty six patients were tested for antinuclear antibody and 42 had this antibody. Cardiovascular and cerebrovascular complications, including hypertension, myocardial infarction (defined according to WHO criteria), angina, and transient ischaemic attacks were present in 57 patients. Eighty three patients were treated with cortisone, and 135 with disease modifying antirheumatic drugs.

Abbreviations: β_2 -AR, β_2 adrenoceptor; CI, confidence interval; OR, odds ratio; PCR, polymerase chain reaction; RA, rheumatoid arthritis; RF, rheumatoid factor; SNPs, single nucleotide polymorphisms

Rheumatoid arthritis (RA) is associated with a number of genetic polymorphisms. The HLA-DRB1 gene is a genetic risk factor in several ethnic groups, and rheumatoid factor (RF) positive destructive joint disease and mild seronegative disease are associated with different HLA-DRB1 alleles.^{1–3} HLA-DP molecules,⁴ genetic variations of the T cell receptor,^{5–8} polymorphisms of the genes coding for tumour necrosis factor α ,^{9 10} interleukin 10,¹¹ and corticotropin releasing hormone¹² are also associated with the disease. Thus, several genetic determinants might add together to confer disease risk.

β_2 Adrenoceptor (β_2 -AR) is present on skeletal and cardiac muscle cells and on peripheral blood lymphocytes and represents a link between the sympathetic nervous system and the immune system. Patients with RA have a decreased number of β_2 -AR on peripheral blood mononuclear cells, and this decrease correlates with disease activity and defective suppressor cell functions.^{13–15} In rats with experimental arthritis, β_2 -AR contributes to the initiation and progression of joint damage.¹⁶ Patients with RA frequently have an abnormal function of the hypothalamic-pituitary-adrenal axis and autonomic nervous system dysfunctions,¹⁷ and impaired control of the immune response through effects on the β_2 -AR by the autonomic nervous system has thus been suggested to be a factor affecting RA.

The gene encoding for β_2 -AR (*ADRB2*) is located at chromosome 5 q31–32.^{18–20} Three single nucleotide polymorphisms (SNPs) at amino acid positions 16, 27, and 164 significantly alter receptor functions. Down regulation

Table 1 Distribution of SNPs at amino acid positions 16 and 27 in patients with rheumatoid arthritis (RA) and in healthy controls (HC)

Amino acid position	RA (n = 154) No (%)	HC (n = 198) No (%)	p	pc	OR	95% CI
Genotype						
ArgArg16	20 (13)	16 (8)	NS			
ArgGly16	80 (52)	69 (35)	0.0016	0.0048	2.02	1.31 to 3.11
GlyGly16	54 (35)	113 (57)	<0.0001	<0.0001	0.41	0.26 to 0.63
GlnGln27						
GlnGlu27	46 (30)	68 (34)	NS			
GluGlu27	85 (55)	77 (39)	0.0026	0.0078	1.93	1.26 to 2.97
	23 (15)	53 (27)	0.0088	0.0264	0.48	0.28 to 0.83
Allele						
Arg	120 (39)	101 (26)	0.0002	0.0004	1.86	1.35 to 2.57
Gly	188 (61)	295 (74)	0.0002	0.0004	0.54	0.39 to 0.74
Carrier						
Arg	100 (65)	85 (43)	<0.0001	<0.0001	2.46	1.59 to 3.80
Non-Arg	54 (35)	113 (57)	<0.0001	<0.0001	0.41	0.26 to 0.63
Gln						
Non-Gln	131 (85)	145 (73)	0.0088	0.0176	2.08	1.21 to 3.59
	23 (15)	53 (27)	0.0088	0.0176	0.48	0.28 to 0.83

β_2 -AR genotyping

The procedures were performed according to Large *et al.*²⁹

Genomic DNA was extracted from leucocytes in samples of whole blood by digestion with proteinase K followed by phenol/chloroform extraction. Polymerase chain reaction (PCR) amplification was performed on an automated apparatus (PTC-200; SDS Co, Falkenberg, Sweden).

PCR amplification of the DNA segment containing codon 27 of the *ADRB2*

PCR amplification of the DNA segment containing codon 27 of the gene was carried out in a volume of 26 μ l containing 300–500 ng DNA, 0.38 mM of each deoxynucleoside triphosphate, 10% buffer (100 mM Tris-HCl, 15 mM MgCl₂, 500 mM KCl, pH 8.3), 10% dimethyl sulphoxide, 20 pmol of each primer, and 0.13–0.63 U of Taq DNA polymerase. The forward primer was 5-GGCCCATGACCAGATCAGCA-3 and the reverse primer was 5-GAATGAGGCTTCAGGCGTC-3. PCR was started with denaturation at 94°C for 4 minutes, followed by 30 cycles of denaturation (94°C, 1 minute), annealing (63°C, 1 minute), and extension (72°C, 1 minute), with a final extension at 72°C for 10 minutes. The PCR product size from these primers is 353 bp. The amplified product was digested at 37°C for 1 hour with 0.4 U of Ita I. The fragments were resolved on a 2% ultrapure DNA agarose gel with Tris-acetate EDTA (40 mM Tris-acetate, 2 mM EDTA) buffer, and visualised under ultraviolet illumination after staining with ethidium bromide. This digestion produced fragments of the following sizes: 27, 55, 97, and 174 bp in Gln27 homozygotes; 27, 55, 97, 174, and 229 bp in Gln27Glu27 heterozygotes; and 27, 97, and 229 bp in Glu27 homozygotes. The 27 bp fragment was too small to be resolved on the gel.

PCR amplification of the DNA segment containing codon 16 of the *ADRB2*

PCR amplification of the DNA segment containing codon 16 of the gene was carried out in the same way as for the codon 27 but using 5-CTTCTTGCTGGCAGCAAT-3 as the forward primer and 5-CCAGTGAAGTGATGAAGTAGTTGG-3 as the reverse primer. The other differences included using an annealing temperature of 56°C and excluding dimethyl sulphoxide. The PCR product size from these primers is 201 bp. The forward primer is complementary to the BAR-2 DNA sequence except for one nucleotide which permits the

creation of a BsrDI restriction site. The amplified product was digested at 60°C for 1 hour with 2 U of BsrDI. The fragments were resolved on a 3% Meta-Phor agarose gel with Tris-borate EDTA (89 mM Tris, 89 mM boric acid) buffer and visualised under ultraviolet illumination after staining with ethidium bromide. This digestion produced fragments of the following sizes: 14, 56, and 131 bp in Arg16 homozygotes; 14, 23, 56, 108, and 131 bp in Arg16Gly16 heterozygotes; and 14, 23, 56, and 108 bp in Gly16 homozygotes.

Statistical analysis

A Mann-Whitney U test was used to compare the values of antibody concentrations and activity of disease between two groups. A χ^2 test with Yates's correction was used for comparing the prevalence of different SNPs. Odds ratios (ORs) and 95% confidence intervals (95% CIs) for relative risks were calculated after use of Fisher's exact test when necessary. A p value <0.05 was considered to be significant. Agreement between the observed genotypes and those predicted by the Hardy-Weinberg equilibrium was assessed by χ^2 test.

RESULTS

The distribution of expected and observed frequencies of different genotypes at amino acid position 16 was according to the Hardy-Weinberg equilibrium in both patients and healthy subjects (p = 0.77 and 0.67, respectively). The distribution at amino acid position 27 was in accordance with this rule in both patients with RA (p = 0.56) and in healthy subjects (p = 0.11).

Table 2 Association between carriage of Arg at amino acid position 16 and carriage of Gln at amino acid position 27 in patients with rheumatoid arthritis (RA) and healthy controls (HC)

	Carriage of Arg16 in RA		Carriage of Arg16 in HC	
	Arg16 ⁺	Arg16 ⁻	Arg16 ⁺	Arg16 ⁻
Gln27 ⁺	100	31	83	62
Gln27 ⁻	0	23	2	51
OR	149.95		34.14	
p Value	<0.0001		<0.0001	

OR, odds ratio.

Table 1 shows the frequencies of different genotypes, alleles, and carrier states at amino acid positions 16 and 27. The prevalence of homozygosity for Gly16 in patients with RA was decreased, and so was the allelic frequency for Gly16. The allele Arg16 and carriage of Arg16 were more prevalent in RA.

Patients with RA had a higher prevalence of GlnGlu27 and a decreased frequency of homozygosity for Glu27. Patients were more often a carrier of Gln27.

Table 2 shows the linkage disequilibria between amino acid positions 16 and 27 in both groups. No patient had a combination of Arg16⁺-Gln27⁻, and this combination was present in only two healthy subjects. This is in accordance with our previous study of patients with myasthenia gravis²³ and found also in patients with hypertension.²⁸ There was a decreased prevalence of the combination Arg16⁻-Gln27⁻ in patients with RA ($p < 0.01$, OR = 0.44, 95% CI 0.25 to 0.77). The concentration of RF and the activity of the diseases were associated with the genotype combination. Patients with genotype GlnGlu27 had more active disease than patients homozygous for Glu27 (mean activity scores 4.8 and 3.9, respectively; $p = 0.0085$). Patients with the genotype combination GlyGly-GlnGlu had significantly higher RF than all the other patients ($p < 0.05$), with a median titre of 1/640 compared with 1/320 for the rest of the patients. These patients also had more active disease (mean activity score 5.1) than those with GlyGly-GluGlu (mean activity score 4.1; $p < 0.05$) or ArgGly-GlnGln (mean activity score 4.3; $p < 0.05$).

Also, the carrier state was associated with disease activity. Patients with Arg⁻Gln⁺ had higher RF than those with Arg⁺Gln⁺ (mean values 1/2047 and 1/1024, respectively; $p < 0.05$) and also significantly more active disease than those with Arg⁻Gln⁻ (mean activity scores 5.1 and 4.1, respectively; $p < 0.05$). Carriage of Gln⁺, including both Arg⁺Gln⁺ and Arg⁻Gln⁺, was accompanied by more active disease (mean activity score 4.7) than non-carriage of Gln (Gln⁻Arg⁻) (mean activity scores 4.7 and 4.1, respectively; $p < 0.05$).

The prevalence of different alleles or genotypes did not differ between patients stratified according to the presence of antinuclear antibody and vascular complications.

DISCUSSION

In this study we have shown a new genetic association with the *ADRB2* on chromosome 5 and shown that RA is associated with carriage of Arg16 (OR = 2.46) and of Gln27 (OR = 2.08). SNPs at amino acid positions 16 and 27 may thus constitute an additional genetic factor contributing to the susceptibility for RA.

Additional associations emerged when patients were stratified according to concentration of RF and activity of disease. A high concentration of RF and high disease activity were associated with the absence of Arg16 and carriage of Gln27. An explanation for this phenomenon might be that the Glu27 type of receptor is present in a less mature form and thus less likely to induce formation of autoantibodies than the Gln27 type of receptor.¹⁹ In asthma, the IgE levels are associated with the SNP at amino acid position 27.²² Further, our earlier study of patients with myasthenia gravis showed that antibodies against β_2 -AR were less common in patients homozygous for Glu27.²³ These patients also had a less severe disease than the others.²³ Thus, these SNPs seem to affect the immune response in the disease, possibly through the receptor on the cells of the immune system.

Our observation that carriage of Arg16 and Gln27 was in linkage disequilibrium confirms earlier studies showing this pattern in both healthy subjects and in patients with asthma.^{25, 32, 33}

The functional significances of the different SNPs are still incompletely known. Down regulation promoted by agonists is enhanced in receptors homozygous for Gly16 and those homozygous for Gln27.¹⁹ β_2 -AR is present on lymphocytes and might thus be of importance for the regulation of the immune responses, both the autoantibody production and the cytokine profile. Our results suggest that the polymorphisms of β_2 -AR may be one contributing factor for the susceptibility to RA and also its severity, although the functional implications are obscure.

The SNPs of the β_2 -AR are associated with asthma,^{22, 24–27} hypertension,²⁸ and obesity.²⁹ β_2 -AR contains significant amounts of N-linked carbohydrate and glycosylation of β_2 -AR determines the surface expression of the receptor.³⁴ Positions 16 and 27 are close to the glycosylation sites and may be important for cellular processing and β_2 -AR expression. How Arg16, which is less common than Gly16, relates to the susceptibility to RA remains to be elucidated. Our finding of linkage disequilibria between SNPs at amino acid positions 16 and 27 is in accordance with the findings of others.³³

Thus, this study of two β_2 -AR SNPs showed that carriage of Arg16 and of Gln27 is associated with RA. Carriage of Gln27 was associated with activity of disease and, in combination with non-carriage of Arg16, with higher levels of RF. The β_2 -AR SNPs may thus constitute an additional non-major histocompatibility complex association in RA.

NOTICE OF DUPLICATE PUBLICATION

We wish to draw readers' attention to significant overlap between this article and one published in the December issue of the *Scandinavian Journal of Rheumatology* (2004;**33**:395–9). The article published in *Scand J Rheumatol* is an earlier version, which differs in certain details from that in *Ann Rheum Dis*. The "duplicate" submission arose from a misunderstanding among the authors.

Kristian Stengaard-Pedersen, editor *Scandinavian Journal of Rheumatology*

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Authors' affiliations

B-Y Xu, L Årle Hag, A K Lefvert, Immunological Research Laboratory, Centre for Molecular Medicine and Department of Medicine, Karolinska Institutet, Stockholm, Sweden

S-B Rantapää-Dahlquist, Department of Rheumatology, University Hospital, Umeå, Sweden

Correspondence to: Professor A K Lefvert, Immunological Research Laboratory, CMM, Karolinska Hospital, S-171 76 Stockholm, Sweden; Ann.Kari.Lefvert@cmm.ki.se

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